Original Article

Intravitreal Bevacizumab Injection Alone or Combined with Triamcinolone Versus Macular Photocoagulation In Bilateral Diabetic Macular Edema; Application of Bivariate Generalized Linear Mixed Model with Asymmetric Random Effects in a Subgroup of a Clinical Trial

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Abstract

Purpose: To compare the efficacy of intravitreal bevacizumab (IVB) injection alone or with intravitreal triamcinolone acetonide (IVB/IVT) versus macular photocoagulation (MPC) in bilateral diabetic macular edema (DME).
Methods: In this study we revisited data from a subset of subjects previously enrolled in a randomized clinical trial. The original study included 150 eyes randomized to three treatment arms: 1.25 mg IVB alone, combined injection of 1.25 mg IVB and 2 mg IVT, and focal or modified grid MPC. To eliminate the possible effects of systemic confounders, we selected fellow eyes of bilaterally treated subjects who had undergone different treatments; eventually 30 eyes of 15 patients were re-evaluated at baseline, 6, 12, 18, and 24 months. Using mixed model analysis, we compared the treatment protocols regarding visual acuity (VA) and central macular thickness (CMT).
Results: Improvement in VA in the IVB group was significantly greater compared to MPC at months 6 and 12 (P = 0.037 and P = 0.035, respectively) but this difference did not persist thereafter up to 24 months. Other levels of VA were comparable at different follow-up intervals (all P > 0.05). The only significant difference in CMT was observed in favor of the IVB group as compared to IVB/IVT group at 24 months (P = 0.048).
Conclusion: Overall VA was superior in IVB group as compared to MPC up to 12 months. Although the IVB group showed superiority regarding CMT reduction over 24 months as compared to IVB/IVT group, it was comparable to the MPC group through the same period of follow up.

Keywords: Asymmetric Random Effects; Bivariate Generalized Linear Mixed Model; Diabetic Macular Edema

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INTRODUCTION

Diabetic macular edema (DME) is the main cause of visual impairment in diabetic patients.[1] Common thinking was that since macular laser photocoagulation (MPC) reduced the possibility of moderate visual loss by 50%, such therapy entails a beneficial effect on DME.[2]

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The Diabetic Retinopathy Clinical Research Network (DRCRnet) reported improvement of more than 5 letters in visual acuity (VA) by MPC at 1, 2, and 3 years of follow-up in 51%, 47%, and 62% of cases, respectively.[3-5] Alternative or adjunct treatments for DME such as anti-vascular endothelial growth factor (VEGF) therapy[3,6-10] and intravitreal triamcinolone acetonide (IVT)[3,11-15] have been evaluated in most recent studies. DRCRnet disclosed that in spite of an early beneficial effect from 4 mg intravitreal triamcinolone (IVT) on retinal thickening and VA at 4 months as compared to 1 mg IVT or focal/grid photocoagulation, final mean VA at 24 and 36 months was better in the MPC group.[4,5] Some reports have shown a promising effect from intravitreal anti-VEGF agents for treatment of DME.[3,6-10,16,17] The VA outcome was significantly better with ranibizumab as compared to MPC for DME at 6 months[17] and 24 months.[18] In a previous trial, we have shown that intravitreal bevacizumab (IVB) injection with or without IVT had a better therapeutic effect on VA in DME in contrast to MPC at 3 months[19] the effect of which was maintained up to 24 months.[20-22]

Some studies have shown that patient characteristics such as age, type of diabetes control and HbA1C levels, in the pre-treatment, treatment and post-treatment period could affect the response to medications used for treatment of diabetic retinopathy (DR).[23-26] Furthermore, other studies have reported the effect of genetic factors and other patient characteristics on the response to intravitreal ranibizumab therapy in age-related macular degeneration (ARMD).[27-29] Thus, comparing the efficacy of various DME treatment protocols, baseline and systemic individual factors should be taken into account. However, as most of these studies are randomized clinical trials, one assumes insufficient sample size for considering all of them.

One possible way to overcome the problem of systemic and baseline individual factors is to compare different treatments in subjects with bilaterally involved eyes, thus patient characteristics which may affect the results are controlled and matched. On the other hand, bilateral eyes are expected to be correlated and frequent measurements also add another level of correlation to these data. There are some statistical methods consider these correlations. The generalized linear mixed models (GLMMs) are well known statistical methods used to handle such data sets.[30] In addition, multivariate mixed models can tackle both the correlation caused by intra-subject organs and repeated measurements.[31-34] Normality distribution of random effects is one of the most important assumptions in these models. Violation of this assumption can result in serious misleading inference.[35-40] Some have suggested random effect models with asymmetric as well as symmetric distribution to avoid such problems.[41-43]

In this study using the bivariate generalized linear mixed model with asymmetric random effects, we attempted to eliminate the role of possible confounders in comparing three treatments for DME and consider the correlation in fellow eyes of the same subject from a subset of patients already reported in a previous paper.[22] Herein, we selected patients with bilateral involvement receiving different treatments for each eye.

METHODS

This clinical trial was approved by the Ethics Committee of the Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences. The trial registration number was NCT00370669. The study adhered to the tenets of the Declaration of Helsinki and informed consent was obtained from all participants. Details of the patients and methods of the trial have been published previously.[19,20,22] The present study is a subgroup analysis of patients with bilateral eye involvement.

This randomized, double-blind clinical trial was performed at Labbafinejad Medical Center between September 2005 and May 2007. All naive eyes with clinically significant DME based on Early Treatment Diabetic Retinopathy Study (ETDRS) criteria were included. Exclusion criteria were previous focal or panretinal laser photocoagulation, history of intraocular injection, glaucoma or ocular hypertension, intraocular surgery, significant media opacity, VA worse than 20/300 or better than 20/40, iris neovascularization, and high risk for proliferative diabetic retinopathy. Other exclusion criteria were pregnancy, monocular status, uncontrolled diabetes mellitus and serum creatinine ≥ 3 mg/dL. Primarily, 150 eyes of 129 patients were enrolled and randomly allocated to one of the three study arms using the random block permutation method with random block lengths of 4 and 6. The study groups were as following: (1) 0.05 mL (1.25 mg) bevacizumab (Avastin; Genentech, Inc., South San Francisco, CA, USA [made for F. Haffmann-La Roche, Ltd., Basel, Switzerland]) injection named as the IVB group; (2) bevacizumab injection plus 0.05 mL (2 mg) triamcinolone (HEXAL Pharmaceuticals, Holzkirchen, Germany) known as the IVB/IVT group, and (3) the MPC group. The fellow eyes in bilateral cases were treated 1-week apart. According to ETDRS criteria and provided that VA was not better than 20/40, retreatment was performed for persistent clinically significant macular edema at 12-week intervals with the assigned initial treatment.

As described in the original protocol,[22] if both eyes of a patient met the inclusion criteria, each eye was randomized individually, hence it was possible for both eyes of bilateral cases to be allocated into the same treatment group. In the present subgroup analysis study, we chose only bilateral cases who underwent different

treatments in fellow eyes.Therefore, only 30 eyes of 15 patients were recruited for this study.

Change in best-corrected visual acuity (BCVA) and CMT measured by optical coherence tomography (OCT) were the primary outcome measures. Using standard Snellen charts, BCVA was recorded in the logarithm of the minimum angle of resolution (logMAR) notations. OCT mapping was achieved by a time-domain device (Zeiss, Dublin, CA, USA). Measurement of retinal thickness was performed in a 3.5 mm diameter circle centered on the fixation point. CMT was measured as mean thickness on the 1-mm circle centered on the fovea.

To preserve investigator masking, procedures were carried out by staff other than the study investigators. A 20 s sham laser procedure was performed using an aiming beam of laser on the macula of eyes in the IVB and IVB/IVT groups. A sham injection was done in the MPC group, with a needleless syringe pressed against the conjunctiva. BCVA measurement and OCT were performed by masked examiners.

Statistical methods

To compare VA and CMT at follow up periods with baseline values within each group, we utilized a general mixed model. In addition, another mixed model was used to compare CMT and VA in the treatment groups after adjustment for baseline values and to consider correlation between fellow eyes of patients at each follow up. Benjamin and Hochberg method was employed for for multiple comparisons.[44] In the last step, to obtain an overall comparison between treatment responses throughout the study duration considering the two levels of correlation in subjects and follow-up, a bivariate generalized linear mixed model with asymmetric random effects was used. P <0.05 were considered as statistically significant. Statistical analysis was performed using R Software (version 3.0.0, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The 24-month results of this trial have been previously published.[22] Out of 21 patients with bilaterally enrolled fellow eyes, 6 patients who had received the same type of treatment in both eyes were excluded. Overall, 15 eligible patients were evaluated at 6, 12, 18, and 24 months, of whom 4 (27%) subjects received IVB in one eye and IVB/IVT in fellow eye; 4 (27%) received IVB in one eye and MPC in the other eye; and 7 (46%) patients received IVB/IVT in one eye and MPC in the fellow eye [Figure 1]. Baseline characteristics of patients who completed the study are summarized

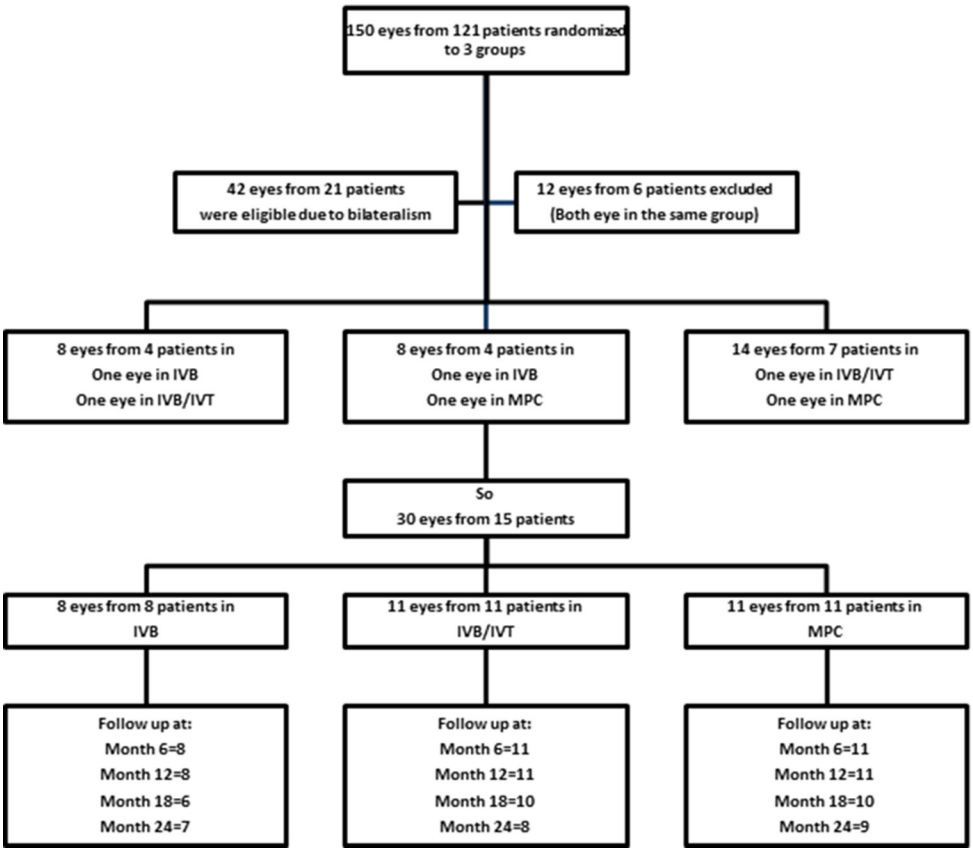

Figure 1. Flowchart for enrolled subjects throughout the trial.

Table 1. Patient characteristics in each combination group

Parameter	Total	IVB+IVB/IVT	IVB+MPC	IVB/IVT+MPC
Sex				
Female/male	5/10	3/1	1/3	1/6
Age (years)				
Mean±SD	61±4	59±3	64±3	60±4
Median (range)	60 (55-67)	60 (55-61)	64 (60-67)	60 (55-66)
Diabetes duration (years)				
Mean±SD	11±3	11±1	13±3	9±2
Median (range)	10 (6-16)	12 (10-12)	13 (10-16)	9 (6-11)
IOP (mmHg)				
Mean±SD	14.2±2.4	14.2±2.9	16±0	13.3±2.3
Median (range)	13.5 (11-18)	14 (11-18)	16 (16-16)	12.5 (12-18)
VA (logMAR)				
Mean±SD	0.63±0.33	0.71±0.37	0.67±0.33	0.55±0.3
Median (range)	0.48 (0.1-1.4)	0.69 (0.22-1.4)	0.54 (0.4-1.3)	0.4 (0.1-1.22)
CMT (μm)				
Mean±SD	328±145	343±170	312±154	329±136
Median (range)	283 (117-651)	261 (195-651)	230 (199-605)	301 (117-556)

IVB, intravitreal bevacizumab injection; IVT, intravitreal triamcinolone injection; MPC, macular laser photocoagulation; IOP, intraocular pressure; VA, visual acuity; logMAR, logarithm of the minimum angle of resolution; CMT, central macular thickness; SD, standard deviation

Table 2. Mean BCVA (logMAR) changes from baseline and median proportions of change for each group during follow-up

	IVB	IVB/IVT	MPC	*P*‡	Multiple comparison
Baseline (mean±SD)	0.84±0.37	0.66±0.29	0.44±0.22		
6 months	0.62±0.28	0.65±0.42	0.66±0.47	0.037	IVB versus MPC
0-6 months	−0.22±0.14	−0.01±0.24	0.23±0.4		
Relative change %	−26	2	52		
P within†	0.027	0.973	0.04		
12 months	0.54±0.33	0.61±0.42	0.56±0.36	0.035	IVB versus MPC
0-12 months	−0.3±0.13	−0.05±0.18	0.13±0.36		
Relative change %	−36	−8	30		
P within†	0.022	0.973	0.179		
18 months	0.69±0.43	0.59±0.44	0.52±0.25	0.117	-
0-18 months	−0.16±0.27	−0.07±0.22	0.08±0.3		
Relative change %	−19	−11	18		
P within†	0.355	0.973	0.339		
24 months	0.81±0.54	0.61±0.46	0.46±0.23	0.444	-
0-24 months	−0.04±0.49	−0.05±0.26	0.02±0.3		
Relative change %	−5	−8	5		
P within†	0.858	0.973	0.79		

‡Adjusted for baseline based on mixed model; †Based on mixed model. Adjustment for multiple comparisons performed by Benjamin and Hochberg method. IVB, intravitreal bevacizumab injection; IVT, intravitreal triamcinolone injection; MPC, macular laser photocoagulation; BCVA, best-corrected visual acuity; logMAR, logarithm of the minimum angle of resolution; SD, standard deviation

in Table 1. Up to the last follow up, retreatment with the assigned regimen was required in all eyes in the IVB, IVB/IVT groups [Table 2]. The mean number of retreatments for each arm was 1.87 ± 0.93, 2.0 ± 1.05 and 1.36 ± 0.76 in the IVB, IVB/IVT and MPC groups, respectively ($P = 0.198$).

Table 2 demonstrates the mean and median proportions of improvement in VA at each follow-up interval in the study groups. The results of mixed effects modeling showed that compared to baseline values, VA improvement was significantly different among the groups up to 12 months ($P = 0.037$ and $P = 0.035$ at 6 and 12 months, respectively) in favor of IVB. Within the groups, analysis showed that VA improvement was significant only in the IVB group up to 12 months ($P = 0.027$ and $P = 0.022$ at month 6 and 12, respectively). In the course of the study, VA improved in the IVB/IVT group and decreased in the

MPC group, although none of these changes reached a significant level [Figure 2]. The percentage of eyes with VA improvement of equal or better than 2 Snellen lines was 38%, 9%, and 18% in the IVB, IVB/IVT, and MPC groups at 12 months, respectively. These percentages were 42.9%, 37.5% and 33.3% in the IVB, IVB/IVT, and MPC groups at 24 months, respectively.

Although VA improvement at 6 and 12 months was significantly in favor of IVB as compared to MPC, the difference did not remain significant thereafter up to 24 months based on intention to treat analysis (P = 0.177 and P = 0.444 at 18 and 24 months, correspondingly). The IVB/IVT group showed better VA improvement than the MPC group, however, this difference was not statistically significant at any follow-up interval (all $P > 0.05$). All comparisons were repeated by per protocol analysis but showed no change appeared in the results. The bivariate generalized linear mixed model showed that the IVB group demonstrated greater improvement in VA (0.33 logMAR, 95% credible interval: 0.23–0.44) as compared to the MPC group and also the IVB/IVT group (0.23 logMAR, 95% credible interval: 0.12–0.33) throughout the study period. On the other hand, the IVB/IVT group showed a negligible difference with the MPC group through the study (0.11 logMAR, 95% credible interval: −0.01–0.20)

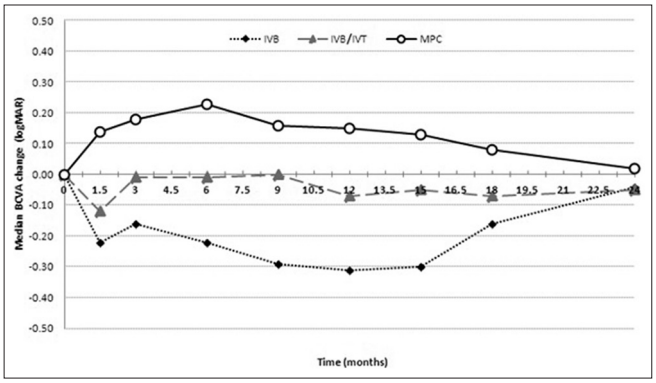

Figure 2. Mean best-corrected visual acuity changes in relation to baseline values in the logarithm of the minimum angle of resolution notations in the three groups at different time points. IVB, intravitreal bevacizumab injection; IVB/IVT, IVB/intravitreal triamcinolone injection; MPC, macular photocoagulation; BCVA, best-corrected visual acuity; logMAR, logarithm of the minimum angle of resolution.

Performing the same comparison for CMT changes, the IVB group had a significantly greater CMT reduction than the IVB/IVT group at 24 months [Table 3, P = 0.048]. Also, CMT had an increasing trend in IVB/IVT group ($P < 0.001$) through the study. The change in CMT of IVB group was different compared to the IVB/IVT group (108 µ, 95% credible interval: 59–157) and MPC group (51 µ, 95% credible interval: 4–100) through the study follow ups. Also, the IVB/IVT group showed some increase in CMT as compared to the MPC group (−57 µ, 95% credible interval: −96 to − 17) [Figure 3].

DISCUSSION

In the current study using the generalized linear mixed model on bilateral DME cases who had received different

Table 3. Mean CMT changes from baseline and median proportions of change for each group during follow-up

	IVB	IVB/IVT	MPC	$P^{\ddagger}$	Multiple comparison
Baseline (mean±SD) (µm)	364±188	324±132	305±131		
6 months	278±115	334±194	327±133	0.462	-
0-6 months	−86±172	10±115	22±53		
Relative change %	−24	3	7		
P within†	0.493	0.899	0.983		
12 months	287±94	380±192	335±142	0.233	-
0-12 months	−77±186	56±125	30±68		
Relative change %	−21	17	10		
P within†	0.558	0.78	0.983		
18 months	340±121	396±190	303±107	0.145	-
0-18 months	−24±96	72±123	−2±97		
Relative change %	−7	22	−1		
P within†	0.783	0.78	0.983		
24 months	302±103	420±215	304±117	0.048	IVB versus IVB/IVT
0-24 months	−62±151	96±147	−1±101		
Relative change %	−17	30	0		
P within†	0.612	0.78	0.983		

‡Adjusted for the baseline based on mixed model; †Based on mixed model. Adjustment for multiple comparisons performed by Benjamin and Hochberg method. IVB, intravitreal bevacizumab injection; IVT, intravitreal triamcinolone injection; MPC, macular laser photocoagulation; CMT, central macular thickness; SD, standard deviation; µm, micron

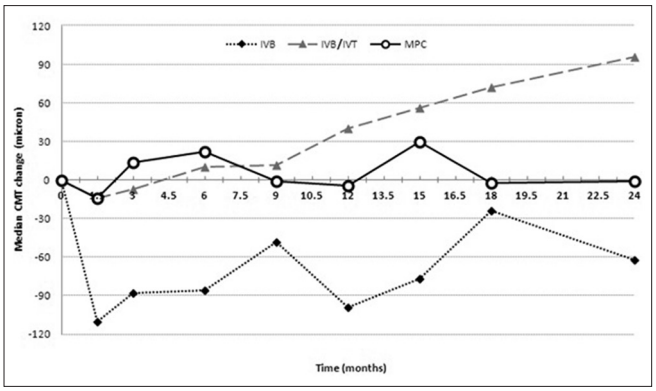

Figure 3. Mean central macular thickness changes in relation to baseline values in micron in the three groups at different time points. IVB, intravitreal bevacizumab injection; IVB/IVT, IVB/intravitreal triamcinolone injection; MPC, macular laser photocoagulation; CMT, central macular thickness.

treatments for each eye, the difference between IVB and MPC at 6 and 12 months was statistically significant in terms of VA improvement. Other intergroup differences regarding VA did not reach a significant level. Considering CMT changes, the difference between the groups was significant only at 24 months in favor of the IVB group as compared to the IVB/IVT group. Moreover, using bivariate generalized linear mixed model with asymmetric random effects, we found that VA improvement was superior in the IVB group as compared to the IVB/IVT and MPC groups and it demonstrated better outcomes regarding CMT as compared to the IVB/IVT and MPC groups through the study.

Comparing these results with those of the original study, a similar pattern of difference was detected. In the original trial, the significant superiority of VA improvement in the IVB group, which had been noted at month 6, was not sustained thereafter up to 24 months and the difference among the groups was not significant at any visit. However, mean VA improvement was greater in the IVB group than the other groups and in the IVB/IVT group as compared to the MPC group. In the current study, conducted only on bilateral treated eyes using different interventions, the difference between IVB and MPC at 6 and 12 months was statistically significant in terms of VA improvement. On the contrary, in the original trial difference in improvement of VA between groups was statistically significant only at 6 months. Participating only bilateral cases in this study along with applying bivariate generalized linear mixed model are possible reasons for the discrepancy between this study and the original one. These two reasons could increase the homogeneity between the groups, decrease the confounding effects of dissimilarities between treatment groups and increase the power of statistical tests. In some other studies, the efficacy of IVB lasted for 6 months[45,46] and its superiority over MPC was reported to persist for 6[17,18,47] and 12 months[3,10] after intervention. However, the effect of IVB and IVB/IVT was not different after 12 months another study.[48]

Concerning CMT changes in this study, the difference among the groups was significant at 24 months in favor of the IVB group in comparison to combined IVB/IVT. We observed CMT reduction of 24% in the IVB group and on the contrary, a CMT increase of 30% in the combined group. However, none of the similar comparisons was statistically significant in the original study.[22] A review of literature showed controversial results regarding the effect of these treatments on CMT; some studies showed that MPC was more effective than anti-VEGF treatments,[17,49] others revealed the superiority of intravitreal injections including IVB alone or combined with IVT as compared to MPC[47] and some were not able to detect any difference.[48]

Our study was powered by eliminating the confounding effect of systemic parameters via comparing two eyes of the same individual receiving two different treatments at the same time. It has been shown that systemic factors such as blood sugar levels, blood pressure, blood lipids, etc., could affect the treatment response.[23,25-29] Keeping all of these parameters under control throughout a study and making them similar among all the groups in a trial are almost impossible practically. Therefore, comparing two eyes of the same subject may compensate for such correlations more ideally. However, as far as intravitreal injections are concerned, the possibility of systemic absorption and fellow eye effect may interfere with the results and can be considered as a source of bias in this study.

A mixed model was used in the present study to compare treatment outcomes; this is a way for intention to treat analysis in clinical trials.[50,51] One of the important assumptions of these models is normal distribution for random effects facilitating their implementation. Simulation studies have shown that although deviation from this assumption does not have a severe impact on the regression coefficient,[38-40] it can affect the variance of their estimation and consequently lead to biased inference.[36,37] We applied a bivariate generalized linear mixed model with asymmetric distribution for random effects to compare the results throughout the study compensating for the correlation of fellow eyes and repeated measurements simultaneously and covering skewed and symmetric distributions for random effects. Despite the small sample size in the current study, which can reduce its power, some new differences were found as compared to the original survey including more prolonged IVB effect on both VA and CMT than other treatments. The justification for this could be simply chance observation. Since all observed differences in the original trial were detected again in this study, it might be concluded that the confounding effects of systemic factors did not significantly impact the previously published results.

In summary, the results of this study using bivariate generalized linear mixed model with asymmetric

random effect on bilateral DME cases reconfirmed the results of our previous clinical trial. In other words, the superiority of repeated IVB injections over combined IVB/IVT and MPC treatment up to only 24 months was reconfirmed. However, this result needs to be proven in larger studies using different treatment agents simultaneously in bilateral DME cases.

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